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Estimates of rhizosphere priming effects are affected by soil disturbance

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ABSTRACT

Laboratory studies have shown that priming effects, caused by inputs of carbon into the rhizosphere, can change the rate of soil organic matter (SOM) decomposition and could have significant impacts on soil carbon cycling. However, there have been few studies in field conditions because of experimental constraints but field data are needed to improve models that forecast the effects of climate change on SOM decomposition rates and the impact of these changing rates on atmospheric CO_2 concentration.

In 2009 a fire at a *Eucalyptus* forest site in Australia killed all standing vegetation. Trenched plots were installed in 2010, approximately 12 months after the fire, and were maintained plant-free for the subsequent year. In 2011, after forest re-growth outside the trenched plots, we compared SOM decomposition rates in the presence of plants (rooted plots) and in the absence of plants (trenched plots) using a natural abundance stable carbon isotope technique with minimal disturbance of the soil. We then compared our results to those obtained in another study conducted at the same time and the same plots using laboratory incubations of sieved soil samples.

There was no difference in SOM decomposition rates between the trenched and the rooted plots estimated using our non-disruptive technique. In contrast, laboratory incubations of sieved soils highlighted a two-fold increase in SOM decomposition rates in the rooted plots compared with rates from the trenched plots. Our results suggest that rhizosphere priming may not actively influence soil carbon turnover in the undisturbed soil environment and question conclusions from laboratory incubation studies. We attribute the different findings from laboratory and field studies to the physical disturbance of the soil involved in laboratory incubations causing the release of previously protected substrates, making them available for decomposition.

1. Introduction

It is estimated that soils globally contain between 2000 and 2400 Pg of organic carbon in the upper 2 m (Jobbágy and Jackson, 2000). Any small change to this vast carbon stock has the potential to affect atmospheric CO_2 concentration significantly and influence the rate of climate change (Houghton, 2007; Paustian et al., 2016). The mechanisms that regulate the accumulation or loss of soil organic carbon are complex, and long-held views and assumptions are being challenged (Schmidt et al., 2011). The mechanisms need to be better understood if we are to improve models and better forecast the impacts of climate change (Davidson and Janssens, 2006).

The interactions between plants and the soil play a central role in the terrestrial carbon cycle. Plants alter the rate and quality of carbon inputs to the soil and exert a strong influence on the soil microbial community (Heimann and Reichstein, 2008; Paterson et al., 2009; Bruggemann et al., 2011). A crucial component of these interactions is the rhizosphere priming effect, defined by Zhu et al. (2014) as the stimulation or suppression of the rate of soil organic matter (SOM) decomposition by the presence of live roots and associated rhizosphere microorganisms compared with the rate of SOM decomposition from rootless soils in the same environmental conditions.

Despite many studies that have observed priming effects in soils incubated in laboratory conditions, and the importance and

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mechanisms regulating priming in the field are poorly understood (Dijkstra and Cheng, 2007; Langley et al., 2009; Bird et al., 2011; Gärdenäs et al., 2011). Many laboratory experiments have used incubations of root-free soils and measured changes in soil CO_2 efflux in response to the addition of labile carbon substrates, such as glucose, used as a surrogate for root carbon inputs (Nottingham et al., 2009; Kuzyakov, 2010). To study the priming effect in the presence of roots and rhizosphere, some laboratory experiments have used isotope labelling methods, whereby soils are sampled, sieved and repacked before being replanted (reviewed by Zhu et al., 2014). However, physically protected SOM is vulnerable to loss when the soil is disturbed by coring and sieving (Zakharova et al., 2015) and the effects can last for periods longer than a year (Zakharova et al., 2014), well beyond the time scale of these priming studies.

Few studies have attempted to estimate priming effects in the field. Estimates are often made from changes in rates of SOM decomposition from long-term measurements of soil carbon made at sites where increased root carbon inputs are inferred from increasing above-ground productivity. This has been done by either using different species (Mazzilli et al., 2014; Tamura and Tharayil, 2014) or artificially increasing atmospheric CO_2 concentration to enhance carbon inputs to soil (Free-air CO_2 Enrichment, FACE) (Langley et al., 2009; Phillips et al., 2012; Sulman et al., 2014). Most results from FACE experiments have shown decreasing carbon content that was attributed to positive priming effects, but some sites showed increasing soil carbon content (Jastrow et al., 2005). Some field studies have shown positive priming effects by directly measuring increases in the rate of SOM mineralisation as a consequence of increasing litter fall on trenched plots (Sulzman et al., 2005; Sayer et al., 2011), but there have been no direct measurements of priming effects due to the presence of the roots and the rhizosphere depositing carbon substrate into the soil.

The lack of studies is probably due to technical difficulties. Inclusion of plants in field studies to address priming effects is challenging, because resolution requires partitioning of the soil CO_2 efflux (R_S) into its heterotrophic component (R_H), derived from SOM turnover, and its autotrophic component (R_A), derived from the respiration of plant roots and associated microbes. Most of the methods that have been used to partition R_S in the field remove the autotrophic component to deduce R_H (Hanson et al., 2000; Kuzyakov, 2006). Studying the influence of the rhizosphere on R_H using such techniques is, by definition, impossible (Subke et al., 2006). In recent years the use of stable isotope analyses that quantify differences in natural ^{13}C discrimination among root and soil carbon pools have allowed measurements of R_H in the presence of the roots and the rhizosphere. Development of an open chamber system to measure and collect the CO_2 emitted from the soil surface (Midwood et al., 2008; Midwood and Millard, 2011) has allowed the isotopic signature of CO_2 respired from the soil ($\delta^{13}CR_S$) to be measured. R_S can then be partitioned into its two components using the $\delta^{13}C$ signature of the CO_2 respired from soil-free roots ($\delta^{13}CR_A$) and root-free soil ($\delta^{13}CR_H$) used as the 'end members' of a simple linear mixing model (Millard et al., 2010; Uchida et al., 2010; Moinet et al., 2016a).

Understanding the mechanisms involved in rhizosphere priming may provide a key to improving models aiming at predicting long-term soil carbon storage (Perveen et al., 2014). There is a need to determine the effects of the physical disturbance of the soil in the methodology employed in most laboratory studies to date. To address this, direct measurements in undisturbed field conditions are needed. Here, we set out to compare results from two different methods. Because the measurements in the studies were made at the same time and locations, any differences in the findings would be attributable only to the methods used. Our study was conducted using a technique involving minimal disturbance to the soil and was compared with the study by Dijkstra et al. (2017) that used laboratory incubations of sieved soil samples, thus involving disturbance to the soil. Therefore, the objective of our study was to assess the effect of this disturbance on the estimates of rhizosphere priming effects.

For both studies, comparison between trenched plots and rooted plots were used to assess the influence of the roots and rhizosphere activities on the rate of soil organic matter decomposition. Trenched plots were established in a *Eucalyptus* forest in Australia following an intense stand-replacing wild fire. Regrowth of active roots was prevented within trenched plots by isolating the soil from the surrounding regenerating forest with plastic membranes (Tang et al., 2005). Two years after the fire, the rhizosphere priming effect was assessed by comparing the rates of R_H in undisturbed soil with and without the presence of the roots and the rhizosphere. We measured the CO_2 efflux within trenched plots to determine 'basal' R_H, without the influence of the rhizosphere. At the same time we used a stable isotope technique to partition R_S in rooted plots in the regenerating forest outside the trenched plots and determined the rate of R_H in soils with active roots and rhizosphere.

2. Materials and methods

2.1. Site description and experimental design

In February 2009 a series of bushfires occurred in the Australian state of Victoria, and a total area of 450,000 ha was burned over a period of several weeks. The study site was located within a 1600 m^2 burnt stand of mountain ash forest (*Eucalyptus regnans* F. Muell.) at the Mount Disappointment State Forest in Victoria, Australia (lat. 37°25S, long. 145°8′E, mean annual temperature 12.4 °C and mean annual precipitation 712 mm). The soils at the site were brown earths (Udic Ustochrept), formed on Mount Disappointment granodiorite formed in the Devonian period (375–341 million years ago) and their characteristics have been described by Adams (1984).

As described by De Rémy de Courcelles (2014), the high-intensity wildfire totally defoliated the crown of the trees and incinerated the litter layer and understorey. Mountain ash are obligate seeders, so the trees were killed by the fire and the stand regenerated from seeds. The first seedlings appeared in October 2009, about 7 months after the fire storm. In March 2010 (just 1 year after the fire), five square trenched plots (1 m^2) were positioned randomly across the study area (Fig. 1). After excavating a trench around the perimeter of each plot to a depth of approximately 600 mm (i.e., below the depth of active root mass and into the sub-soil), a 1 mm thick PVC membrane was installed around the entire perimeter of each plot and the trench was then back-filled to keep the membrane in place. During installation of the PVC membrane, care was taken not to disturb the soil within the plot. After installation, the membrane protruded above the soil surface to a height of approximately 100 mm. All the plants (predominantly *E. regnans* seedlings) were removed by hand at monthly intervals from within the trenched plots. After establishment of the trenched plots, there was rapid and dense regrowth of *E. regnans* seedlings (mean height 2 m, tree number 50–100 seedlings per m^2) outside of the trenched plots (Fig. 1). The experimental set-up was also described by Dijkstra et al. (2017).

Two months before the start of the experiment (January 2011), four collars made from PVC drainpipe (diameter100 × depth 50 mm) were positioned to a depth of 25 mm in the trenched plots (four collars per plot, a total of 20 collars). A further four collars were positioned in the surrounding regenerating forest, within a 5 m radius of the centre of each trenched plot (four collars per plot, a total of 20 collars), defining five rooted plots. The soil CO_2 efflux and rates of heterotrophic respiration were measured in early autumn (between 3 and 8 March 2011).

2.2. Soil CO_2 efflux

Soil CO_2 efflux was measured simultaneously at the four collars within each trenched and surrounding rooted plots using an open chamber system, as described previously (Midwood et al., 2008; Midwood and Millard, 2011). Briefly, the CO_2 concentration in each

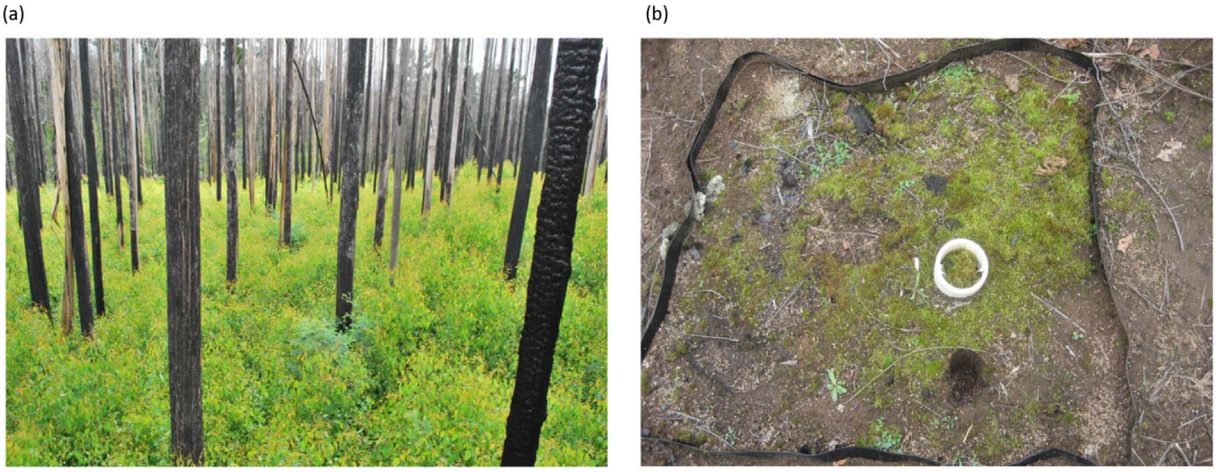

Fig. 1. (a) Mountain ash (*Eucalyptus regnans* F. Muell.) sapling regeneration after two years under the stems of mature trees killed by the fire at Mount Disappointment, Victoria, Australia; and (b) a 100 mm diameter respiration collar placed in a trenched plot.

chamber was maintained slightly above atmospheric concentration by balancing the air outflow from the chamber with the inflow of CO_2 free air, supplied from a cylinder of compressed gas on site (from which CO_2 was removed using soda lime). Chambers were left in place for a minimum period of 1.5 h to allow the efflux and its isotopic signature to recover from any disturbance effect caused by placement of the chamber onto the soil collars. The soil CO_2 efflux was recorded, and was based on the flow rate of CO_2 free air into the chamber required to maintain the desired CO_2 concentration. This rate corresponded to the rate of 'basal' R_H in the trenched plots and to the sum of heterotrophic and autotrophic respirations (R_S) in the rooted plots.

2.3. Stable isotope measurements and partitioning

Immediately after recording soil CO_2 effluxes, samples of air were collected simultaneously from the four chambers into 2 L air-tight Tedlar™ bags. The contents of these bags were then analysed for $^{13}CO_2$ signature (corresponding to $\delta^{13}CR_H$ for the trenched plots and $\delta^{13}CR_S$ for the rooted plots). Immediately after collecting the air, root samples were collected from the rooted plots to a depth of 300 mm, using a stainless steel soil corer (80 mm diameter). Each soil core was broken apart quickly and the roots were removed by hand and transferred to an air-tight Tedlar™ bag. The air in the bags was then evacuated repeatedly and the contents flushed with CO_2-free air and then filled with approximately 0.6 L of CO_2-free air and the contents left to incubate. After 20 min, an aliquot of gas was removed to confirm that the CO_2 concentration fell within 380–450 μmol mol^{-1} using a portable gas analyser (EGM-4, Hansatech Instruments Ltd., King's Lynn, Norfolk, UK). By maintaining the CO_2 concentration within this range, the precision and accuracy of the isotope analyser and measurements of $\delta^{13}CR_A$ were optimised. If the CO_2 concentration within a bag was too dilute, the sample was incubated for longer; if too concentrated, the sample was diluted with CO_2-free air. Incubation times were kept as short as possible to avoid any potential shifts in $\delta^{13}C$ signature that may be caused by changes to the substrates being metabolised in the roots (Millard et al., 2008; Snell et al., 2014).

The proportion of R_S attributable to R_H (fR_H) within the rooted plots was calculated using the following isotope mass balance calculation:

$$fR_H = 1 - \left(\frac{\delta^{13}CR_S - \delta^{13}CR_H}{S^{13}CR_A - \delta^{13}CR_H}\right) \quad (1)$$

where fR_H is the proportion of total efflux attributable to R_H, and $\delta^{13}CR_S$ and $\delta^{13}CR_A$ are the isotopic compositions of the CO_2 effluxes from rooted soils and of the CO_2 respired by excised roots, respectively. $\delta^{13}CR_H$ was the mean value of isotopic composition of the CO_2 effluxes for all replicate measurements in the trenched plots.

Isotope analysis of the air collected from the chambers was conducted on-site using a wavelength-scanned cavity ring-down spectrometer (Picarro G1101-i, Santa Clara, California, USA). To optimise the performance of the analyser in the field, a calibrated gas standard was analysed at the start and end of each day during the campaign, and all subsequent sample analyses were corrected to the mean of these two calibration gas results; the largest correction was + 1.16‰ and the smallest − 0.02‰. An in-line magnesium perchlorate water trap (changed daily) was used to ensure the water content of the gas entering the spectrometer remained below 0.01% (vol/vol).

Soil volumetric water content (θ_S) and soil temperature (T_S) in the upper 100 mm of the soil profile were measured following each measurement of R_S using a Delta-T Moisture probe (Delta-T Devices Ltd., Cambridge, UK) and a 107 thermistor (Campbell Scientific Ltd., Loughborough, UK), respectively.

2.4. Statistical analyses

The effects of root exclusion on R_S, $\delta^{13}CR_S$, $\delta^{13}CR_A$, θ_S and T_S were examined using the PROC TTEST statement in SAS® (version 9.2; SAS Institute Inc., Cary, NC). The folded F test was used to confirm homogeneity of variance. A Bayesian modelling approach described by Brewer et al. (2005) was used to estimate uncertainties around the proportions fR_H and around estimates of R_H in the rooted plots. Essentially, the model used was based on a version of the mass balance given in Eq. (1), where the values of $\delta^{13}CR_S$ are modelled as a weighted sum of end member samples ($\delta^{13}CR_H$ and $\delta^{13}CR_A$). The estimate and uncertainty of R_H are then derived directly from the estimated proportions and the soil surface CO_2 efflux values (R_S).

3. Results

The mean soil surface CO_2 efflux (R_S) measured using the open chamber system was significantly lower in the trenched plots compared to the values for the adjacent rooted plots, with an average difference of 1.8 μmol m^{-2} s^{-1} (Table 1). There were no significant differences in soil temperature or water content between the trenched and rooted plots, and Dijkstra et al. (2017) reported no differences in carbon content. Our incubations showed that across all five sites the mean of $\delta^{13}CO_2$ from roots ($\delta^{13}CR_A$) was − 27.0 ± 0.2‰, and that the root contribution to the CO_2 emitted from the soil surface in the rooted plots resulted in $\delta^{13}CR_S$ being significantly more depleted than the $\delta^{13}C$ value of CO_2 emitted from the soil surface in the trenched plots (which is the value of $\delta^{13}CR_H$ used in Eq. (1), Table 1).

Table 1
Effects of root exclusion on soil CO_2 efflux (Rs), $\delta^{13}CO_2$ of the soil efflux ($\delta^{13}CR_S$) and roots ($\delta^{13}CR_A$), soil volumetric water content (θ_S, depth 0–100 mm) and soil temperature (T_S, depth 0–100 mm) in the trenched and rooted plots. The value of $\delta^{13}CR_S$ in the trenched plots is the value of $\delta^{13}CR_H$ used in Eq. (1). There were significant differences between trenched and rooted plots ($p < 0.05$) for R_S and $\delta^{13}CR_S$, but not for T_S and θ_S. All values are means ($n = 5$), ± 1 standard error shown in brackets. NA = non-applicable.

	R_S (μmol m^{-2} s^{-1})	$\delta^{13}CR_S$ (‰)	$\delta^{13}CR_A$ (‰)	T_S (°C)	θ_S (%)
Trenched	1.7 (0.2)	− 20.7 (0.4)	NA	15.9 (0.7)	22.6 (1.7)
Rooted	3.6 (0.3)	− 24.0 (0.2)	− 27.0 (0.2)	15.3 (0.9)	20.7 (1.1)

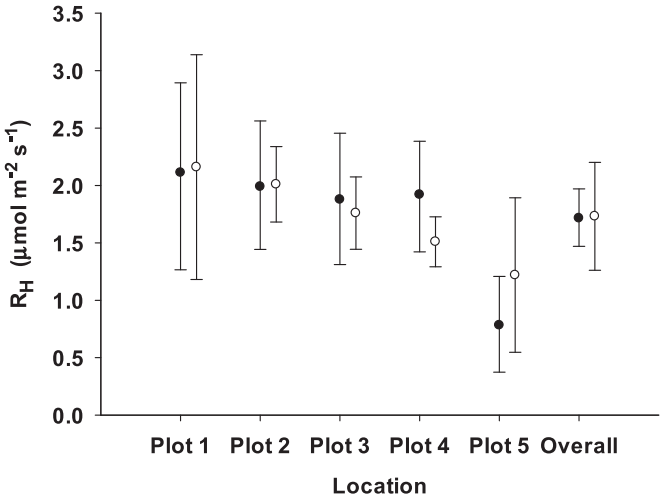

Fig. 2. Estimates of soil heterotrophic respiration (R_H) measured on the trenched plots (open symbols) and for the rooted plots (solid symbols). Errors for the rooted plots are predicted by Bayesian modelling. Error bars are 95% confidence/prediction intervals ($p \leq 0.05$).

For all measurement in the rooted plots, the value $\delta^{13}CR_S$ was more enriched than the value of $\delta^{13}CR_A$ and more depleted than the mean value of $\delta^{13}CR_H$ in the trenched plots. Soil heterotrophic respiration rates measured using the isotopic method in the rooted plots were between 0.8 and 2.1 μmol m^{-2} s^{-1}. This range of values was similar to that of 'basal' R_H; that is, the CO_2 efflux measured on the trenched plots, which varied from 1.2 to 2.2 μmol m^{-2} s^{-1}. To further improve our estimates of R_H in the rooted plots and provide a measure of uncertainty, the Bayesian modelling approach provided an overall estimate for R_H across the site and values at each of the five plots we sampled, together with 95% prediction intervals (Fig. 2). The uncertainty around our estimates of R_H in the rooted plots was ± 15% at the site level, and the predicted efflux of 1.7 ± 0.06 μmol m^{-2} s^{-1} (mean ± standard error) was almost identical to and not significantly different from the efflux rate measured in the trenched plots (1.7 ± 0.17 μmol m^{-2} s^{-1}, Table 1), where the overall variation was ± 22%.

4. Discussion

At our site we combined a trenched plot approach with a natural abundance ^{13}C technique to quantify the rhizosphere priming effect in field conditions directly. By making measurements at the same time as those using laboratory incubation of sieved soils sampled from the same trenched and rooted plots (Dijkstra et al., 2017), we were able to compare results from two different techniques and assess the effect of soil disturbance involved in the laboratory incubation technique. A frequent criticism of the trenching method, applicable to both studies by Dijkstra et al. (2017) and ourselves is that in establishing such plots, any roots present are killed and provide new substrates to decomposers. Because these new substrates decay, the values of R_H from trenched areas could be a biased estimate of the rate of R_H in the rooted plots (Hanson et al., 2000; Ngao et al., 2007; Moinet et al., 2016b). There are limited data available on the decomposition rates of dead root material in field conditions, and there is no evidence that all dead root material had decomposed at the time of measurements, even two years after a fire. However, in this study we selected a site that had experienced an intense forest fire, killing all the canopy trees and removing all of the undergrowth. We installed the trenched plots after this event. Therefore, any ongoing decomposition of dead root material would have contributed to the CO_2 efflux in both trenched and rooted plots, so the additional effect of living roots and their inputs of carbon on the decomposition of SOM could be resolved.

Another assumption in our approach was that the isotopic composition of heterotrophic CO_2 in our rooted plots was identical to that measured in the trenched plots. We reasoned that SOM takes many years to accumulate, and so the vast majority of carbon present would pre-date the establishment of the new saplings and would be the primary source of R_H. The total carbon content of the soil at this site was measured as 10.7% (mass basis) (De Rémy de Courcelles, 2014), and no significant differences were found between trenched and rooted plots at the time of our study (Dijkstra et al., 2017). It is thus unlikely that carbon inputs from the newly established *Eucalyptus* saplings would have had sufficient time to affect the substantial carbon reserve and change the isotopic signature of the R_H component significantly.

In Australian *Eucalyptus* forests it is known that seedlings germinate profusely on bare soil surface after a fire. This is primarily due to the presence of a nutrient-rich ash bed providing a source of readily available nitrogen and phosphorus, and a lack of competition from other plants (Attiwill, 1994; Launonen et al., 1999). Our own measurements (data not reported here) indicated that, two years after the fire, the number of saplings was between 0.5 million and 1 million stems per hectare. We speculated that the dense, live-root network created by the *Eucalyptus* saplings would be a potential site for active priming, significantly modifying SOM turnover rates. Laboratory studies have shown priming effects to change R_H dramatically, up to > 200% (Paterson and Sim, 2013; Zhu et al., 2014). This was also the observation made by Dijkstra et al. (2017) who reported that rates of respiration from sieved soils sampled in the rooted plots were more than twice those from the trenched plots at the same time when our measurements were made. These authors also showed a significantly greater microbial biomass in the presence of plants, resulting in increased nitrogen mineralisation used for plant growth. At our site, litter decomposition was clearly present and would also have been expected to stimulate fast-growing microbes that decompose litter, potentially producing extracellular enzymes, leading not only to the metabolism of fresh material but also soil organic matter that could lead to priming effects (Fontaine et al., 2004). The drivers of priming have been linked to the available microbial biomass and available nutrient supply (Kuzyakov, 2010), and it is likely that the activity of decomposers was increased at our site in the rooted plots.

We did not detect any significant differences in R_H between the rooted plots and the trenched plots and, consequently, we found no evidence for rhizosphere priming at the time of measurements, positive or negative. It is possible that the rhizosphere priming effect was relatively small, only contributing marginally to net carbon turnover, and so undetectable by our isotopic method. Using a Bayesian modelling approach we were able to calculate prediction intervals by plot and overall values for the entire site. Our range in estimates of R_H across the entire site was ± 15% (0.27 μmol m^{-2} s^{-1}), and this value was close to that for the predicted value of R_H. This value represents a combination of uncertainties, introduced by our isotope measurements and natural biological variation across the site. Disentangling these two sources of uncertainty is not possible in this study, and would need

further work in a more controlled environment where the biological and environmental variations could be minimised. However, it is clear that the overall error was relatively small, and so the magnitude of any priming effects must have been equally small at the time of measurements.

So, if microbial activity was indeed higher in the rooted plots and led to a strong increase in respiration rates from incubated sieved soil samples (Dijkstra et al., 2017), why did it not result in an increase in R_H in undisturbed field conditions? It is well known that a large proportion of SOM may be protected from decomposers through (i) physical segregation into aggregates where oxygen and enzyme diffusion are limited and (ii) chemical binding by adsorption onto mineral surfaces (Jastrow et al., 1996; Sollins et al., 1996; Six et al., 2002; Conant et al., 2011). There is also increasing evidence that these mechanisms may be predominant in regulating the rate of SOM decomposition in the undisturbed soil environment (Davidson and Janssens, 2006; Dungait et al., 2012; Lehmann and Kleber, 2015). In laboratory incubations on sieved soils, at least part of that protection is likely to be compromised due to physical disturbance, releasing significant amount of substrates which become available for decomposition (Zakharova et al., 2014, 2015). In the laboratory incubation conducted by Dijkstra et al. (2017), the higher microbial biomass in the soils from the rooted plots is likely to have resulted in faster rates of mineralisation of these newly released substrates. It is also likely that these substrates were protected in the undisturbed soil and were therefore unavailable for decomposition. Notwithstanding this, the higher microbial biomass in the rooted plots must have had access to additional supplies of carbon to sustain growth. We argue that the carbon inputs from plants and litter alone were providing this supply. The increase in soil CO_2 efflux in the rooted plots compared to that in the trenched plots could thus be a combination of 'apparent priming' effect, as defined by Kuzyakov (2010), in addition to root respiration.

Part of the SOM is thought to be unprotected (Six et al., 2002) and could have been the subject of positive priming in the presence of the increased microbial biomass. It is important to note that our natural abundance stable isotope technique separates the heterotrophic and autotrophic fluxes based on the isotopic signature of the CO_2 respired by roots and root-free soils when they are physically separated. It is possible that a recently deposited pool of SOM in the rhizosphere results in a relatively depleted ^{13}C signature. Decomposition of this recent SOM pool by heterotrophic organisms would thus contribute to the autotrophic component and be accounted for as autotrophic respiration. From our data we are unable to speculate whether or not priming of such a depleted pool of SOM contributed to the higher soil CO_2 efflux in the rooted plots.

5. Conclusions

Using a natural abundance isotopic technique in undisturbed field conditions, we found no evidence for a change in the rate of decomposition of SOM due to the presence of roots. This finding was in contrast to results from laboratory incubations of sieved soils samples carried out at the same time from the same rooted and trenched plots and showing a strong positive priming effect. We attribute this difference to the physical protection of a large pool of SOM that prevents access by the microbial biota in undisturbed conditions and its release when soil samples are removed and sieved for laboratory studies. Our study was conducted on a one-time set of measurements and although we are not able to infer the existence of an overall priming effect at this site at other times in the year with different biotic and abiotic conditions, our findings question the conclusions from studies that have used disrupted, sieved soils to test for the existence and magnitude of the rhizosphere priming effect. We argue caution is needed if priming effects are included in models that forecast long-term changes in soil carbon stocks with changes in vegetation dynamics and climate. More experimental work is needed to determine changes in the physical protection of SOM when investigating the rhizosphere priming effects at different sites and on different timescales.

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